

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re patent application of Aviv Shaish

Serial No. 10/668,601

Group Art Unit: 1654

Filed: 09/24/2003

Examiner: M.C. Flood

For: THERAPEUTIC USES OF DUNALIELLA POWDER

DECLARATION
under Rule 132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Aviv Shaish, an Israeli citizen residing at Thalmei Yehyel 200, Israel, hereby declare:

1. I am currently a Scientist in Sheba Medical Center.
2. My list of publications is attached herewith as Annex A. My fields of expertise include Carotenoids and Atherosclerosis.
3. I carried out experiments to test the effect of *Dunaliella* on diabetes. Female db/db mice (n=20) were divided into 2 groups (n=10 in each group). 10 mice were fed chow diet alone (group A) and 10 mice were fed chow diet fortified with *Dunaliella* powder (group B) (8% of the diet, weight/weight). After 8 weeks group A was divided into 2 sub-groups:
 - (a) **Control**- was continued to be fed chow diet, and
 - (b) **Rosiglitazone**- was treated with a low dose of the thiazolidinedione Rosiglitazone (0.005% of the diet, weight/weight).
4. Group B was also divided into 2 sub-groups:
 - (a) ***Dunaliella***- was continued to be fed chow diet with *Dunaliella* powder, and
 - (b) ***Dunaliella* + Rosiglitazone** - was treated with *Dunaliella* powder and a low dose of Rosiglitazone.

5. Results

In the *Dunaliella* + Rosiglitazone treated group, glucose tolerance (area under curve) improved significantly, $P=0.005$ and $P=0.028$ as compared to the Control and Rosiglitazone treated groups, respectively (Figs. 1 A,B). Moreover,

fasting plasma glucose was lowered in the *Dunaliella* + Rosiglitazone group as compared to the other groups (Fig. 2). However, this change did not reach significance.

6. To study the effect of high-dose rosiglitazone plus *Dunaliella* on diabetes, 40 mice db/db mice were divided to 4 groups (n=10 in every group) and treated for 4 weeks by different treatments:

- a. control (chow diet)
- b. Rosiglitazone (chow diet + Rosiglitazone 0.02%)
- c. *Dunaliella* (chow diet + *Dunaliella* 8%)
- d. Rosiglitazone plus *Dunaliella* (chow diet + Rosiglitazone 0.02% + *Dunaliella* 8%).

7. The combination treatment of High-dose rosiglitazone plus *Dunaliella* did not improve the effect of High-dose rosiglitazone on diabetes in db/db mice (see fig. 3). In contrast, the combination treatment of Low-dose rosiglitazone plus *Dunaliella* had a beneficial effect on diabetes in db/db mice compared to low-dose rosiglitazone treatment alone (see Fig. 1a).

8. In summary, the addition of *Dunaliella* powder to treatment of diabetes with Rosiglitazone allows a lowering of the dose of the drug, thus reducing the adverse side-effects of Rosiglitazone.

9. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

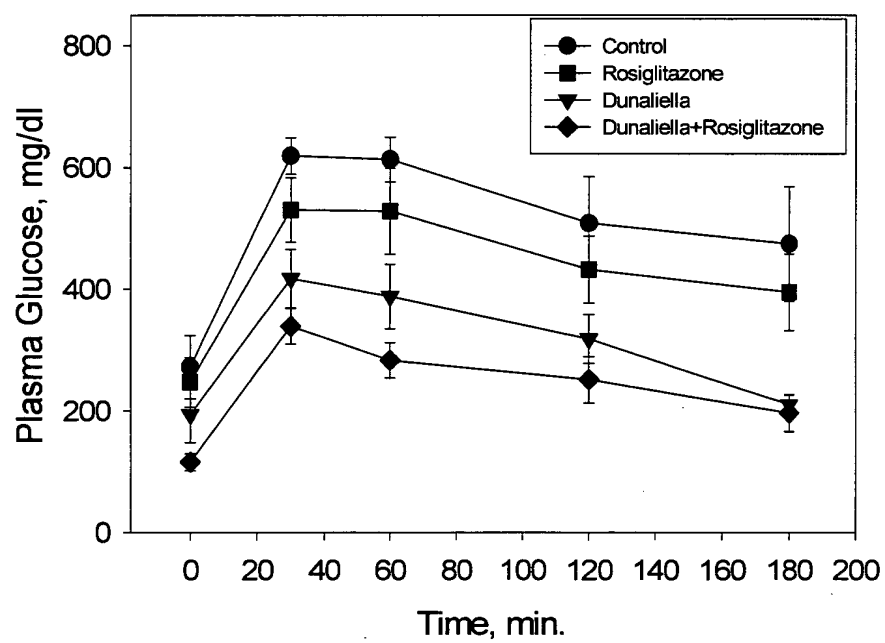
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Aviv Shaish

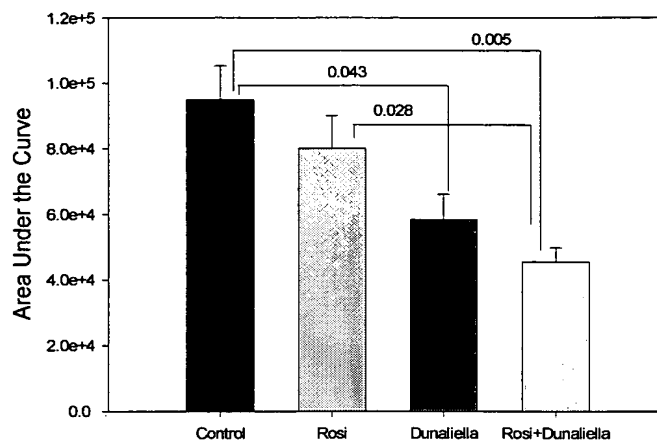
Dr. Aviv Shaish



Fig. 1A: Glucose tolerance test



**Fig 1B. area under curve of
glucose tolerance test:**



**Fig 2.: Plasma fasting
glucose levels after 15 days
of treatment.**

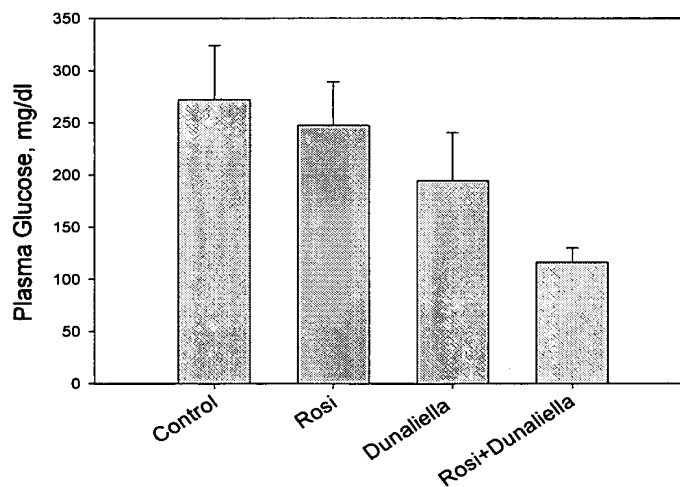


Fig. 3

